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Unconventional bacteria in urinary tract disease: *Gardnerella vaginalis*

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Unconventional bacteria in urinary tract disease: *Gardnerella vaginalis*. Bladder aspirate urine samples (N = 190) were cultured for the presence of fastidious microorganisms. These samples were obtained from patients with urinary tract disease in whom standard bacteriologic investigation had failed to indicate infection. *Gardnerella vaginalis* was recovered alone, or in association with *Ureaplasma urealyticum* from the bladder urine of 33% of patients with reflux scarring. *G. vaginalis* was localized to the upper urinary tract in 75% of these patients with bladder counts greater than 10^3 colony-forming units per milliliter. The results show that two fastidious microbial species, not conventionally associated with urinary tract infection, are recoverable from the upper urinary tracts of patients with so-called sterile pyelonephritis.

Une bactérie inhabituelle dans les uropathies: *Gardnerella vaginalis*. Des échantillons urinaires obtenus par aspiration vésicale (N = 190) ont été mis en culture pour rechercher des micro-organismes fragiles. Ces échantillons ont été obtenus chez des patients ayant une uropathie, chez qui une étude bactériologique standard n'avait pas mis d'infection en évidence. *Gardnerella vaginalis* a été retrouvée seule, ou en association avec *Ureaplasma urealyticum* dans l'urine vésicale de 33% des malades qui avaient des lésions de reflux. *G. vaginalis* était localisée dans le tractus urinaire supérieur chez 75% des malades avec des comptes vésicaux supérieurs à 10^3 unités par millilitre. Ces résultats indiquent que deux espèces microbiennes fragiles, non habituellement associées à l'infection urinaire, peuvent être retrouvés dans le tractus urinaire supérieur de malades ayant une pyélonéphrite dite stérile.

Despite many studies, the relationship between urinary tract infection and the progressive loss of renal function in reflux nephropathy remains unresolved. Whereas Ransley, Risdon, and Path [1] have shown that under experimental conditions atrophic pyelonephritic scarring only develops in association with the intra-renal reflux of infected urine, patients with bilateral chronic pyelonephritis may progress to endstage renal failure in the absence of infection [2]. However, most studies have been based on the culture of urine for the limited range of bacteria that are conventionally associated with urinary tract infection. We have shown [3] that *Ureaplasma urealyticum*, a venereally acquired mycoplasma is recoverable from the bladder urine and renal parenchyma of patients with reflux nephropathy. The present report communicates findings related to a second fastidious micro-organism, *Gardnerella vaginalis*, in this patient group.

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Methods

Study group. We examined 190 patients referred for consultation with a range of urinary tract diseases (Table 1). A midstream urine culture for conventional urinary pathogens in each patient failed to yield a significant microorganism. Patients did not receive antibiotics during the 72-hr period prior to urine collection. Samples from 35 apparently healthy adult females without a history of urinary tract disease were examined as controls.

Urine collection. Urine was collected from the bladder by suprapubic aspiration (SPA) as described previously [3]. Samples were drawn into sterile 20-ml syringes which were capped with sterile needles and sent to the laboratory.

Anaerobic culture. Urine and 10^{-2} saline dilutions of urine in volumes of 0.1 ml were spread over the surface of prereduced agar medium in an anaerobic chamber. Plates were incubated anaerobically (85% N_2, 10% H_2, 5% CO_2) for up to 7 days.

Aerobic culture. Blood agar medium was inoculated as above in a laminar flow cabinet and incubated aerobically (95% air, 5% CO_2) for 72 hr.

Liquid culture. Urine samples in volumes of 0.1 and 1.0 ml were added to 10 ml of chopped meat medium. After 3, 10, 17 and 24 days of incubation, broth media were subcultured aerobically and anaerobically with blood agar medium. Subculture plates were incubated for 7 days.

Culture media. Samples were cultured on Columbia agar containing 4% defibrinated horse blood, 0.5 μg/ml vitamin K, and 5 mg/liter hemin. After a number of patients yielded a growth of *G. vaginalis*, the human blood agar medium of Greenwood et al [4] was also used.

Microbial identification. Isolates were identified according to a standard scheme [5] and in the patients with catalase-negative coryneform bacteria, the criteria of Dunkelberg, Skaggs, and Kellog [6] and Bailey, Voss, and Smith [7] were used.

Level of colonization of urinary tract. The probable site of colonization of the urinary tract was determined by the bladder washout method of Fairley as previously described [3].

Leukocyte count. The degree of pyuria was quantified by counting leukocytes in the urine sediment (concentrated 20-fold by centrifugation) with a Fuchs-Rosenthal counting chamber.

Results

Microorganisms were cultured from SPA urine samples of 56 out of 190 (29%) patients and 3 out of 35 (9%) control subjects

Table 1. Frequency of isolation of *G. vaginalis* from patients classified according to their clinical diagnosis

Diagnostic group	Number patients tested			Number yielding *G. vaginalis*			Number yielding *G. vaginalis* and *U. urealyticum*		
	Female	Male	*Total*	Female	Male	*Total*	Female	Male	*Total*
Chronic atrophic pyelonephritis (reflux nephropathy)	33	5	*38*	11	0	*11*	9	0	*9*
Recurrent urinary tract infection (normal IVP)	18	0	*18*	3	0	*3*	2	0	*2*
Renal papillary necrosis (analgesic nephropathy)	8	3	*11*	1	0	*1*	1	0	*1*
Renal calculi	9	7	*16*	0	0	*0*	0	0	*0*
Glomerulonephritis (biopsy proven)	43	34	*77*	5	0	*5*	3	0	*3*
Medullary or polycystic kidney disease	5	4	*9*	1	0	*1*	0	0	*0*
Miscellaneous renal disease not included above	13	8	*21*	1	0	*1*	0	0	*0*
TOTAL	*129*	*61*	*190*	*22*	*0*	*22*	*15*	*0*	*15*

Table 2. Frequency of isolation of microorganisms from bladder aspirate urine of 190 patients and 35 control subjects

	Gardnerella vaginalis	*Escherichia coli*	*Streptococcus faecalis*	*Streptococcus mitior*	*Staphylococcus saprophyticus*	*Lactobacillus brevis*	*Eubacterium lentum*	*Ureaplasma urealyticum*	No growth
Patients	22[a]	2	6	4	1	1	1	34	134
Control subjects	2	0	0	0	0	0	0	2	32

[a] Data represent the number of subjects.

(Table 2). Thirteen patients gave a growth of conventional urinary pathogens and two yielded anaerobic species. A further 22 patients harbored *G. vaginalis* and in 15 of these, *U. urealyticum* was also present. Nineteen other patients had *U. urealyticum* alone. Of the 35 control subjects, two exhibited a growth of *G. vaginalis* and in one of these, *U. urealyticum* was also present. A further subject harbored *U. urealyticum* alone. The findings related to *U. urealyticum* in this patient group were the subject of an earlier communication [3] and will not be considered further in this report.

Colony counts. Colony counts from 10 out of 13 patients with conventional urinary pathogens were less than 10^4 cfu/ml (Table 3) with cfu representing colony-forming units. Of the two patients carrying an anaerobic species, one had 500/ml *Lactobacillus brevis* and the other had greater than 10^5/ml *Eubacterium lentum.* In contrast, 17 out of 22 patients with *G. vaginalis* harbored greater than 10^5 cfu/ml in the bladder.

Aspects of colonization of the urinary tract by G. vaginalis

Isolation from different patient groups. Table 1 shows that *G. vaginalis* was only recovered from the bladder urine of female subjects, that is, in 22 out of 129 female and 0 out of 61 male patients ($P < 0.01$). Whereas the frequency of isolation from the total female patient group was not significantly different from the control group, a significantly increased frequency was observed in female patients with reflux nephropathy ($P < 0.01$). Negative findings in the other five patient groups provided a second "control" group with which to compare the reflux nephropathy group ($P < 0.01$).

Level of urinary tract involvement. Of the 22 patients with *G. vaginalis* in SPA urine, 12 gave evidence of upper tract involvement and infection was confined to the bladder in seven patients. In three patients, localization results could not be interpreted with confidence due to low numbers of bacteria in the bladder urine. Nine of 12 patients with upper tract involvement had reflux nephropathy. None of these patients had evidence of persisting vesico-ureteric reflux on a dehydrated micturating cystourethrogram.

Association with pyuria. Urine leukocyte counts were raised (> 2000 cells/ml) in one of seven patients with *G. vaginalis* alone and four of 15 patients yielding both *G. vaginalis* and *U. urealyticum.*

Association with squamous epithelial cells. An invariable finding on microscopy of SPA urine from culture-positive patients was the presence of squamous epithelial cells covered with bacteria (Fig. 1). "Cobblestone" areas of stratified epithelium were observed at cystoscopy in each of four patients examined.

Discussion

The present study was undertaken to determine whether microorganisms not conventionally associated with urinary tract infection were recoverable from the bladder urine of patients with urinary tract disease. In a previous report [3] it

Table 3. Colony counts of several bacterial species in bladder aspirate urine

Colony count, *cfu/ml*	*G. vaginalis*	*E. coli*	*S. faecalis*	*S. mitior*	*S. saprophyticus*	*L. brevis*	*E. lentum*
$> 10^5$	17[a]		1				1
10^4 to 10^5	2		1		1		
10^3 to 10^4	3		2	2			
10^2 to 10^3		1	2	2		1	
10^1 to 10^2		1					
Total	*22*	*2*	*6*	*4*	*1*	*1*	*1*

Abbreviation: *cfu*, colony-forming units.
[a]Data represent the number of patients.

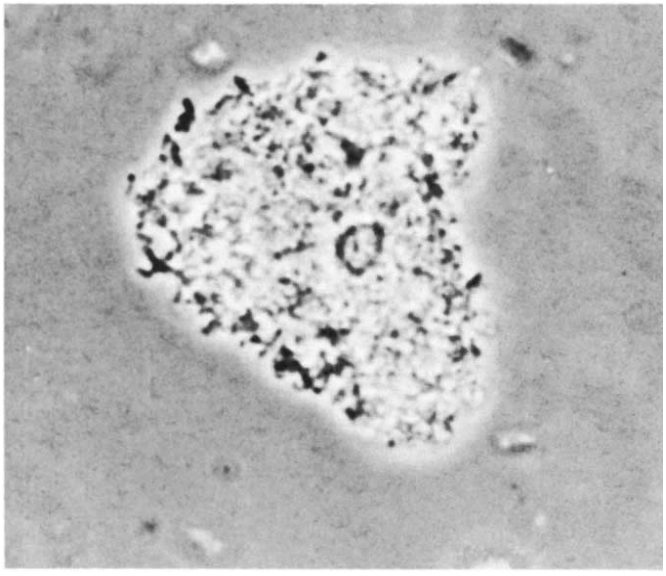

Fig. 1. *Bacteria-laden squamous epithelial cell from bladder aspirate urine.* (Phaco ×132)

was shown that some patients with reflux nephropathy harbored *Ureaplasma urealyticum* in the bladder and upper urinary tract and the present results show that *Gardnerella vaginalis*, another fastidious species, was also recoverable from the upper urinary tract in this patient group.

G. vaginalis was originally isolated from the genital tracts of men with prostatitis and women with cervicitis [8] and from the vaginal discharge of women with "non-specific" vaginitis [9]. Most subsequent clinical and experimental interest in this microorganism has been directed toward assessing its pathological significance in the genital tract. The first report of *G. vaginalis* in the urinary tract was by McFadyn and Eykyn [10] who recovered these bacteria from SPA samples of 159 of 1000 healthy pregnant women. This pattern of recovery in pregnancy has been confirmed subsequently by McDowall et al [11] who isolated *G. vaginalis* from SPA urine of 18% of a group of healthy pregnant women and 58% of pregnant women with underlying renal disease. Whereas only a limited number of patients with urinary tract infection involving *G. vaginalis* in non-pregnant patients has been reported from other centers [12–14], we have cultured these bacteria from the bladder urine of renal transplant recipients [15] and patients with acute urinary symptoms [16, 17]. In the present study *G. vaginalis* was recovered alone or in association with *U. urealyticum* from the bladder or upper urinary tract of 11 of 33 females with reflux nephropathy and 11 of 96 females with other urinary tract diseases. In addition, two of 35 apparently healthy female subjects were carrying *G. vaginalis* in the bladder. Bacteriuria with conventional urinary pathogens occurs in 2 to 5% of asymptomatic females [18]. In this small study in which SPA urine from only 35 healthy subjects was examined, three (8.6%) harbored fastidious species and none had conventional urinary pathogens. This suggests that asymptomatic bacteriuria involving fastidious species might be more prevalent than bacteriuria among females due to conventional urinary pathogens. The pathological significance of *G. vaginalis* in the bladder is unknown at present and it is possible that colonization of the bladder by these bacteria, as with conventional urinary pathogens, may be a benign finding in some individuals. This is in accord with the view of McFadyn and Eykyn [10] who considered that *G. vaginalis* was of no pathological significance in their pregnant population. It is interesting to note in their study, however, that the frequency of symptomatic infection with conventional pathogens at a later stage of pregnancy was significantly higher in the group carrying *G. vaginalis* in the bladder [19].

In the present study, *G. vaginalis* was cultured from the bladder urine of female patients only. Phase-contrast microscopy of the urine sediment from culture-positive individuals showed numerous bacteria adherent to squamous epithelial cells. These bore a close resemblance to the "clue" cells which are considered pathognomic of *G. vaginalis* infection in the vagina. This suggests that in both sites, *G. vaginalis* readily attaches to squamous epithelial cells. Vaginal inclusion epithelium in the region of the bladder trigone is a normal finding in postpubertal females in whom the squamous epithelium of the vagina usually covers the trigone and floor of the urethra [20, 21]. It follows that the ability of *G. vaginalis* to attach to urogenital squamous epithelium might provide an effective mode of spread from the vagina and might also determine the site of primary colonization within the bladder. Similar areas of squamous epithelium are not seen in the male bladder and it is possible that this difference in embryologic development might explain our failure to recover *G. vaginalis* from the bladder urine of any of the 61 males examined.

The finding that *U. urealyticum* accompanied *G. vaginalis* in the bladder urine of 15 out of 22 patients may be of significance for the disease-causing potential of these fastidious bacteria in the urinary tract. A similar association between these species has also been observed in the bladder urine of renal transplant

patients [15], patients with acute urinary symptoms [16, 17] and pregnant women [11]. It is uncommon to isolate multiple microbial species from patients with conventional urinary infections. However, these fastidious microorganisms appear to have a lower potential for tissue invasion than conventional urinary pathogens, and it is possible that mixed colonization might assist one or both species to become established in the urinary tract. In this regard it was noticeable that neither of these species was regularly associated with other bacterial species in the bladder urine.

Thirteen of the 56 culture-positive patients harbored conventional urinary pathogens, which might be considered of interest since all patients were culture-negative on standard bacteriologic testing of midstream urine. In 12 of 13 patients, however, SPA counts were less than 10^5 cfu/ml, and it would not have been possible to diagnose bacteriuria in these patients using the criteria of Kass [22] for midstream urine cultures. This further emphasizes the value of suprapubic aspiration of urine for the investigation of symptomatic patients with equivocal midstream urine cultures.

Despite many studies, the role of anaerobic bacteria in urinary infection remains controversial. The finding that obligate anaerobes were cultured from only two patients in this study lends support to the view that these microorganisms are only of limited significance in urinary tract disease. By contrast, *G. vaginalis,* a fastidious capnophilic species, was considerably more prevalent in our patient group. Yet to date, little attention has been given to the natural history of this microorganism in the urinary tract. There are several factors which may have contributed to this situation. *G. vaginalis* is a capnophilic species and growth usually develops on appropriate media in a carbon dioxide-supplemented aerobic atmosphere. However, six of 22 isolates in this study failed to grow under these conditions on primary isolation and subculture. Because the reason for this phenomenon could not be ascertained during the course of the study, carbon dioxide-supplemented anaerobic incubation was preferred. When midstream urine samples are cultured anaerobically on enriched blood agar media for *G. vaginalis,* a profuse growth of commensal microflora develops, and it is often difficult to distinguish *G. vaginalis* from other coryneform (diphtheroid) species. In this regard it is probably significant that most relevant studies, to date, have used the culture of bladder aspirate urine.

The findings presented in this report show clearly that a significant number of patients with so-called "sterile pyelonephritis" yielded a growth of bacteria from bladder aspiration samples when appropriately cultured. The most frequently isolated species were fastidious microorganisms unconventionally associated with urinary tract disease. In the light of these findings further study of the natural history and pathologic significance of *G. vaginalis* and *U. urealyticum* in the bladder and upper urinary tract is warranted. These results also emphasize the need to include culture methods that will grow these microorganisms before statements can be made that urine samples from patients with chronic atrophic pyelonephritis are sterile.

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